REMARKS

Status of the claims:

With the above amendments, claims 14, 20, 24, 25, and 26 have been amended. Claims 14-26 are pending and ready for further action on the merits. No new matter has been added by way of the above amendments. Support for the amendment to claim 14 comes from the parent PCT document (WO 99/67211). The amendment to claim 20 has support at page 19, line 28 as well as the reaction scheme shown in Figure 1. The amendment to claim 26 has support in original claim 14. Reconsideration is respectfully requested in light of the following remarks.

Specification

The Examiner has objected to the lack of an abstract. Attached to the back of this reply, please find an abstract. The Examiner has also objected to Titles in the specification. The Titles in the written description have been amended to conform to US practice. Withdrawal of the objections is respectfully requested.

Rejections under 35 USC §112, second paragraph

Claims 14-26 are rejected under 35 USC §112, second paragraph as being indefinite.

The Examiner asserts that the use of the phrase "such as" in claims 14 and 25 render these claims indefinite because it is not know if the subject matter subsequent to these phrases are part of the claims.

Claim 14 has been amended so that "in a suitable solvent such as acetonitrile" has been deleted. Accordingly, the rejection is moot. Withdrawal of the rejection is respectfully requested.

Regarding claim 25, the language "which may have usual heteroatoms such as S, O, N or P" has been amended to recite "which optionally contains the heteroatoms S, O, N, and P". It is believed that with this amendment that the rejection has been obviated. Withdrawal of the rejection is respectfully requested.

The Examiner has also rejected claim 14 as being indefinite for use of the phrase, "addition of lithium hydride and transfer of the 25-hydroxy group into the lithium alcoholate and subsequent reduction of the nitrile group with lithium aluminum hydride". The phrase has been amended to recite "addition of lithium hydride and conversion of the 25-hydroxy group into the lithium alcoholate and subsequent reduction of the nitrile group with lithium aluminum hydride". In step b) of claim 14, lithium hydride is added and the 25 hydroxy group converted into the corresponding lithium alcoholate. The word "transfer" is an

unfortunate English translation of the German word "überführt" (see WO 99/67211, which is in German, page 7, line is) which can mean both "transfer" or "conversion". One of skill in the art would know immediately that the word conversion is the correct translation of the German word "überführt". Attached to this response, please find the page from the German priority document with this phrase. It is believed that with this change that the rejection has been obviated. Withdrawal of the rejection is respectfully requested.

The Examiner has also rejected claim 15 as being indefinite for use of the phrase "FITC substituted tyrosine". The Examiner asserts that it should be replaced with "FITC and substituted tyrosine". This rejection is traversed.

First, the claims are part of the specification and thus any language that occurs in the claims as originally filed is given the same priority date as anything that occurs in the written description.

Second, FITC stands for "fluorenyl isothiocyanate", which is the commonly used reactive form of fluorescein. FITC conjugation (for instance with amino acids) is a standard reaction in this field. FITC is a standard abbreviation, as evidenced by the more than 10,000 hits this abbreviation recalls when using the search engine Google. Withdrawal of the rejection is respectfully requested.

Claim 20 has been rejected for reciting "3-amino propyl-25-hydroxy-" and "3-amino propyl-1α-25-dihydroxy". This rejection is traversed for the following reasons. Claim 20 has been amended to recite "3-amino propylether-25-hydroxy-" and "3-amino propylether-1α-25-dihydroxy", respectively. It is believed that with this amendment that the rejection has been obviated. Withdrawal of the rejection is respectfully requested.

Claim 26 has been rejected as being dependent upon two different claims (the compound of claim 25 and the method of claim 14). All of the method steps in claim 14 have been incorporated into claim 26. Thus, claim 26 is now only dependent from claim 25. Withdrawal of the rejection is respectfully requested.

Rejections under 35 USC §102

Claims 21-24 have been rejected under 35 USC §102(e) as being anticipated by Holick '127 (WO 97/24127). It is assumed that the Examiner meant to reject claims 21-24 under 35 USC §102(a) as being anticipated by Holick '127 or alternatively under 35 USC §102(e) as being anticipated by Holick '779 (US Patent No. 5,981,779). Nevertheless, this rejection is traversed for the following reasons.

The Examiner asserts that Holick discloses vitamin D compounds that can be used to assay for vitamin D derivatives and points to the whole document.

With regard to Holick '127 (WO 97/24127) and Holick '779 (US Patent No. 5,981,779), it is noted that Holick '779 contains subject-matter that has been added after the filing and publication date of the underlying WO 97/24127 (same as PCT/LTS96/20341).

The "added matter" of Holick '779 is not "general added matter" but refers specifically to the compound provided by the method of the present invention. The title compounds of example 1 and the process of the present invention are "25-hydroxy vitamin-D_3-3β-3'[6-N-biotinyl)-hexamido]amido, propylether" (see formula II on page 7 in the present invention) and the corresponding 1α25-hydroxy vitamin-D derivative.

These compounds are characterized by

(i) a 25-hydroxy vitamin D portion

(ii) a biotin group

(iii) a spacer of defined length between (i) and (ii).

Holick '127 does not disclose formula II of the present Invention.

Example 1 in Holick '127 does not refer to hydroxy vitamin D but to vitamin D. Example 2 refers to a compound having a much

shorter spacer. Moreover, the shown "biotin groups" are not biotin but another hetero ring system. In examples 3 to 5 the so-called biotin group is again not biotin and the shown formulae are all wrong. Holick '779 (the US patent) attempted to correct these errors that appear in Holick '127 (the WO publication). Attached to this response, please find a comparison between the biotin shown in the instant invention and the biotin found in Holick '779.

Most importantly, neither Holick '779 nor Holick '127 contain an enabling disclosure to prepare the claimed 25-hydroxy vitamin-D derivatives. For use as a tracer in a competitive binding assay for measuring the concentration of 25-hydroxy vitamin D in a sample it is of utmost importance that the test compound (tracer) has indeed the 25 hydroxy group. None of the US Patent Nos. cited on page 12, lines 15 to 22 (Holick '127) or of column 3, lines 50 to 55 (Holick '779) disclose a method of preparing 3-amino propylether-25-hydroxy vitamin D or 3-aminopropylether-1α, 25-dihydroxy vitamin D.

The structural analysis of Holick '127 for the claimed target compounds all contain no proof that the claimed compounds were actually synthesized and the 25-hydroxy group was present in any one of the starting or target compounds. There is no proof in Holick '127 or Holick '779 that the 25 hydroxy group was actually present in any one of the starting compounds

(examples 2 to 5) or in any one of the target compounds. Table I at page 21 of the present invention shows that the synthesized target compound contains two types of NH or OH protons and the various NH protons of the biotin ring system. Thus, the NMR spectrum of table 1 of the present invention is in conformity with the structure of a biotin conjugate of 25 hydroxy vitamin D.

Moreover, according to example 6 of Holick '127, compound C was approximately eleven (11) times less efficient in displacing ^{3}H-25-OH-D_3, bound to hDBP, than 25-OH-D_3. This displacement efficiency indicates that no true "compound C" was added in this example because the displacement efficiency for compound C (as synthesized by the present invention) is one (1). In other words, if compound C is added for displacement of ^{3}H-25-OH-D_3 from DBP, then approximately one molecule of compound C is able to displace one molecule of ^{3}H-25-OH-D_3. A displacement efficiency of eleven (11) molecules of compound C will replace one molecule of ^{3}H-25-OH-Vit. This is clear proof that compound C of Holick '127 had no 25-hydroxy group and that another vitamin-D-derivative was added to displace 25-hydroxy vitamin D.

Further, attached please find Scratchard-Plots comparing the displacement efficiencies of biotin-vitamin D (a 25 hydroxy vitamin D compound prepared according to claim 14 and example 1 of the description), vitamin D dimer (a 25 hydroxy vitamin D

dimer as claimed, in claims 25 or 26) and ^{3}H-25-OH-Vit-D. The comparative binding analyses were done as described in example 3 and 4 of the description using vitamin D binding protein from goat serum (see example 3(iv), line 18 on page 24 in the instant invention). The R^2 value of the slope or gradient of the displacement gives the displacement efficiency of the tested compound and is close to 1 for compounds prepared according to the invention. The displacement efficiency is the key parameter for a reliable and true quantization of 25-hydroxy- and 1α,25-dihydroxy vitamin D metabolites in a sample. Consequently, the method of obtaining the vitamin D derivative is also crucial for the method of quantitative detection of 25-hydroxy- and 1α,25 dihydroxy vitamin D metabolite in a sample.

In Applicants' opinion, Holick '127 and Holick '779 provide no evidence that any one of the compounds claimed was actually synthesized and tested. The allegedly tested compound C was by no means the compound claimed because it could not properly displace the corresponding tritiated compound from vitamin D binding protein (DBP). The displacement of the natural vitamin D from its binding protein was the primary purpose for the synthesis of compound C and the results of example 6 of Holick '127 and Holick '779 show that it could not have been compound C (this is examples 6 or 7 in the Holick references). In other words, the displacement of example 6 and the ELISA of example 7

merely describe an unspecific dirt effect obtained by adding a biotin-labeled vitamin-D-like compound, and not the compounds of the instantly rejected claims.

Moreover, example 9 of Holick '127 allegedly produces a biotin conjugate of 1α,25-dihydroxy-vitamin D (compound G) in which the biotin conjugate is attached to $1,25(OH)_2D_3$ via a long "tether". The ether is in fact an "ester chain" (see Figure 6 and compound G) which is attached *in vivo* by esterases regularly present in body fluids such as serum so that the title compound (G) is labile and can by no means be suitable for quantitative determination of a dihydroxy vitamin D compound in serum or other body fluids (see Figure 6 of Holick '127).

It was, in particular, an object of the present invention to provide derivatives of vitamin D, which are also stable with respect to serum enzymes such as esterases (see page 4, line 30 of the English text). This was accomplished in the instant invention but not in either of the Holick references.

In essence, the disclosure of Holick '127 or Holick '779 are both not enabling because both documents do not disclose how or where the starting compound 25-hydroxy vitamin D-3-aminopropyl ether was obtained. 25-Hydroxy vitamin D-3-aminopropylether is not available commercially (and was not at the time of filing either of the Holick references nor any time before) and no synthesis is given in any one of the cited

documents. The results of the displacement experiments also show that the added compounds were not the added title compounds. The alleged displacement results with the fluorescein vitamin D derivative were probably obtained with the corresponding more easily obtained ester compound (see also example 9).

Consequently, the methods of claims 21 to 24 must be considered new compared to Holick '127 and Holick '779. The instant invention has considerable advantages over Holick '127 and Holick '779, namely an eleven times greater sensitivity compared to compound C, and the advantage, which cannot be proven of course, that truly 25-hydroxy vitamin D is indeed competed out from DBP and its concentration measured in a sample. The disclosures of Holick '127 and Holick '779 omit crucial steps so that one of skill in the art would consider them to be a theoretical work enriched by examples 6 and 7, wherein a dirt effect is described.

As an example, none of the biotin groups shown in the description represent the correct structure of biotin. The source of the crucial starting compound 25-OH-D_3-3-aminopropylether is not given and the latter is masked by excessive literature citations. In example 4 of Holick '127 or Holick '779, the vitamin-D aminopropylether derivative, which is extremely difficult to isolate (if possible at all), is

allegedly added in excess to a commercially available compound to synthesize compound F. No reasonable chemist would use a compound that is difficult (if not impossible) to isolate in excess in the next reaction. Moreover, the NMR data and the UV spectra refer to parts of the molecule that do not change and are not the parts that would allow one to differentiate between the molecules. Finally, the biological data (ELISA and displacement experiment) prove that Holick '127 failed to synthesize the claimed title compounds. If a fluorescein conjugate of vitamin D was ever synthesized and tested as claimed in example 6, it likely was the corresponding ester, which is relatively easy to obtain.

For the above reasons, neither Holick '127 nor Holick '779 can anticipate the instant invention. Withdrawal of the rejection is warranted and respectfully requested.

With the above remarks and amendments, it is believed that the claims, as they now stand, define patentable subject matter such that a passage of the instant invention to allowance is warranted. A Notice to that effect is earnestly solicited.

If any questions remain regarding the above matters, please contact Applicant's representative, T. Benjamin Schroeder (Reg. No. 50,990), in the Washington metropolitan area at the phone number listed below.

Attached hereto is a marked-up version to show changes made.

Pursuant to 37 C.F.R. §§ 1.17 and 1.136(a), Applicant(s) respectfully petition(s) for a three (3) month extension of time for filing a reply in connection with the present application, and the required fee of $460.00 is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

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Attachments:
Page 7 of WO 99/67211
Scratchard-Plots indicating "bioactivity with C"
Drawings of Biotin
Abstract

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE WRITTEN DESCRIPTION:

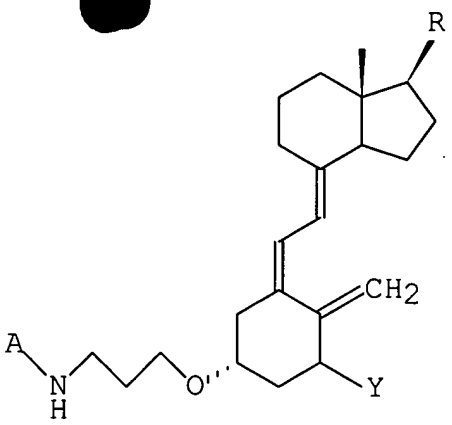
The paragraph starting on page 13, line 17 has been amended as follows.

--After conventional preparation, the 25-OH vitamin D-3β cyanoethylether is mixed with lithium hydride and the 25-hydxoxy group [transferred] converted into the lithium alcoholate. There follows a reduction of the nitrite with $LiAlH_4$, to 25-OH-vitamin-D-3β-3′ -amino propylether. This step is quantitative, without by-products arising. Finally there is effected, if necessary, a biotinylation with an active biotinylation reagent such as LC-BHNS (biotinyl -N-ε-amino caproyl-hydroxy-succinimide ester). The resulting spacer group X has, corresponding to the amino caproyl chain, a length of about 0.8 to 0.9 nm.--

IN THE CLAIMS:

The claims have been amended as follows.

14. (Amended) A method [Method] of obtaining a vitamin D [derivative] compound of the formula:



wherein:

R represents a 25-hydroxy side-group of vitamin D_2 or of vitamin D_3;

Y represents hydrogen or hydroxy;

A represents a functional group, coupled via a spacer group, which can be bound by a protein with high affinity;

characterised by the steps;

a) cyanoethylation of the 3-hydroxy group of a vitamin D starting compound [in a suitable solvent (such as acetonitrile)] in the presence of potassium hydride and tertiary butanol;

b) addition of lithium hydride and [transfer] conversion of the 25-hydroxy group into the lithium alcoholate and subsequent reduction of the nitrile group with lithium aluminum hydride; and

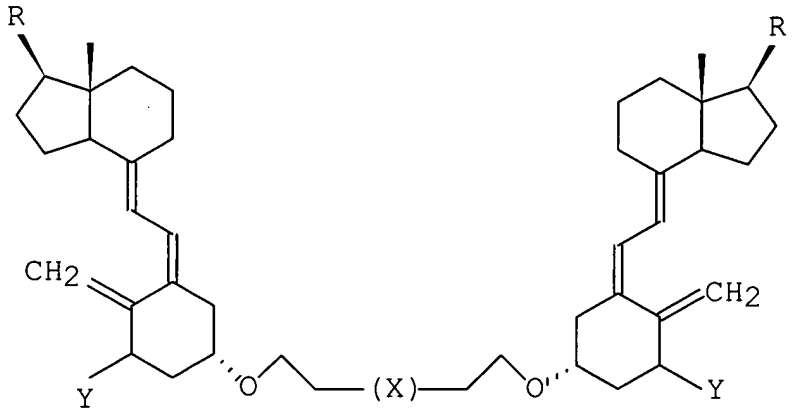
c) linking a spacer group together with a functional group A on the amino propylether side chain.

20. (Amended) Method of producing the 3-amino propylether-25-hydroxy- or 3-amino propylether-1α,25-dihydroxy

vitamin D intermediate compound, characterised by the method steps a) and b) according to claim 14.

24. Reagent kit for the detection of 25-hydroxy- and 1α,25-dihydroxy vitamin D metabolites, characterised in that it contains a standardized quantity of solid or solution of a vitamin D-derivative which is manufactured in accordance with claim[s] 14.

25. (Amended) Vitamin [D-derivative] <u>D-compound</u> of the formula:



wherein;

R represents a 25-OH side group of vitamin D, or

Y represents hydrogen or hydroxyl and

X represents a substituted or non-substituted hydrocarbon group of 0.8 to 4.2 nm length, [which may have usual heteroatoms such

as S, O, N or P] which optionally contains the heteroatoms S, O, N, and P.

26. (Amended) Vitamin D [derivative] compound according to claim 25, obtained by

a) cyanoethylation of the 3-hydroxy group of a vitamin D starting compound in the presence of potassium hydride and tertiary butanol;

b) addition of lithium hydride and conversion of the 25-hydroxy group into the lithium alcoholate and subsequent reduction of the nitrile group with lithium aluminum hydride; and

c) linking a spacer group together with a functional group A on the amino propylether side chain [method according to claim 14] wherein in step c) two vitamin D aminopropyl compounds are coupled by means of condensation with a dicarboxylic acid.